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Agricultural Research

A Priceless Collection

Ask someone to name the world's greatest collections, and they will probably mention France's Louvre, the Soviet Union's Hermitage, and the United States' Smithsonian Institution.

But there are many other collections of equal importance to life on Earth, and one of these is the ARS Culture Collection at the Northern Regional Research Center (NRRC), Peoria, Ill. This collection can best be viewed with a microscope.

Putting an assortment of microorganisms and a small group of scientists and technicians on a par with three of the world's greatest museums may seem to be an overstatement. But in the process of fermentation, bacteria, molds, yeasts, and other organisms are able to transform chemically almost every compound on Earth—animal, vegetable, or mineral—and produce a different compound that may be useful to society.

The ARS Culture Collection preserves more than 75,000 pure, viable cultures of yeasts, molds, bacteria, and other important microorganisms. But that is not all. Each bacterium has about 3,000 genes, and each single yeast cell contains about 10,000 to 25,000 genes. Keeping in mind the opportunities for genetic manipulation offered by recombinant DNA technology, multiply the number of genes times the number of cultures in the collection, and there is a nearly infinite potential for directing the actions of microorganisms to purposes of use to humankind.

The ARS Culture Collection is the largest collection of agriculturally and industrially important microorganisms in the world. These microorganisms are the biochemist's toolmakers, and the tools they make are enzymes—biochemical catalysts that modify and convert existing compounds into different ones.

What separates the Culture Collection's 75,000 organisms from all others found in nature is that the lab's 12 scientists and technicians and their predecessors are devoted to finding out everything there is to know about where the organisms grow naturally, how to maintain and grow them in the laboratory, and what they might be useful for.

In 1904, USDA microbiologist Charles Thom (1872-1956) began the collection with a group of molds, mostly *Aspergilli* and *Penicillia*, when he was assigned to study the behavior of certain cheese-making microorganisms at the experiment station in Storrs, Conn. In 1913, Thom moved his research and the collection to Washington, D.C., where he was assisted by microbiologist Margaret B. Church. The collection became known as the Thom and Church Collection.

When the Northern Regional Research Center opened in 1940, 1,200 mold strains of the Thom and Church Collection were selected to be the nucleus of the Center's Culture Collection Section.

Since then, other collections have been added to the original one, greatly expanding the range of potential purposes to which the microorganisms might be put.

If the possibilities for genetic recombination within the Collection seem inestimable, so, too, does the value of the Collection's accomplishments over the years. To cite just a few examples—

- In 1940, NRRC scientists began looking for a way to produce itaconic acid by fermenting carbohydrates with different strains of the mold *Aspergillus terreus*. Itaconic acid is important in manufacturing plastics, lubricants, and other chemicals, and the system that was developed to produce it is now regularly used throughout industry.
- In 1943, a survey initiated in the Culture Collection eventually found a strain of the mold *Penicillium chrysogenum* that could produce penicillin in a submerged fermentation process in vats instead of on the surface of liquids in trays and pans. This discovery revolutionized the pharmaceutical industry, making large-scale production of antibiotics possible. This breakthrough came at a crucial time in history—during World War II.
- In 1943, a yellow-color variant observed in a culture of the mold *Ashbya gossypii* was found to produce riboflavin, or vitamin B_2. A species of *Streptomyces* (a type of bacterium) was discovered to produce vitamin B_{12}. Fermentation processes were then developed for producing both vitamins in quantity.
- Also in 1943, the bacterium *Leuconostoc mesenteroides* was isolated from spoiled root beer and found to produce the polysaccharide, dextran. This research led to dextran's being tested and then adopted for use as a blood plasma extender in medicine. A search of the Culture Collection for microorganisms that produce polysaccharides led to 96 bacterial strains being identified as dextran producers by 1954.
- In 1956-59, salt resistance was discovered in a water-thickening polysaccharide gum produced by the bacterium *Xanthomonas campestris*. This gum was later named xanthan gum, and now is used as a thickening agent in foods and for recovering more petroleum from pumped-out wells.
- In 1980, studies of the Collection's cultures contributed to the development of standards and analytical techniques to protect human and livestock health by preventing the contamination of foods and feeds by aflatoxin and other mycotoxins.

These are but a few of the many useful discoveries stemming from long and painstaking research in the Culture Collection. Studying these microorganisms and unlocking their secrets, like perusing museum rooms and tapping the knowledge stored there, is a powerful tool for understanding and improving the quality of life.

J.M.R.

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Cover: Photomicrograph of *Pachysolen tannophilus*, a unique yeast that converts xylose (wood sugar) directly into ethyl alcohol. Selected from the ARS Culture Collection at the Northern Regional Research Center, Peoria, Ill., *Pachysolen* could potentially add 4 billion gallons annually to the production of fuel alcohol in the United States. Article begins on p. 8. (PN-6850)

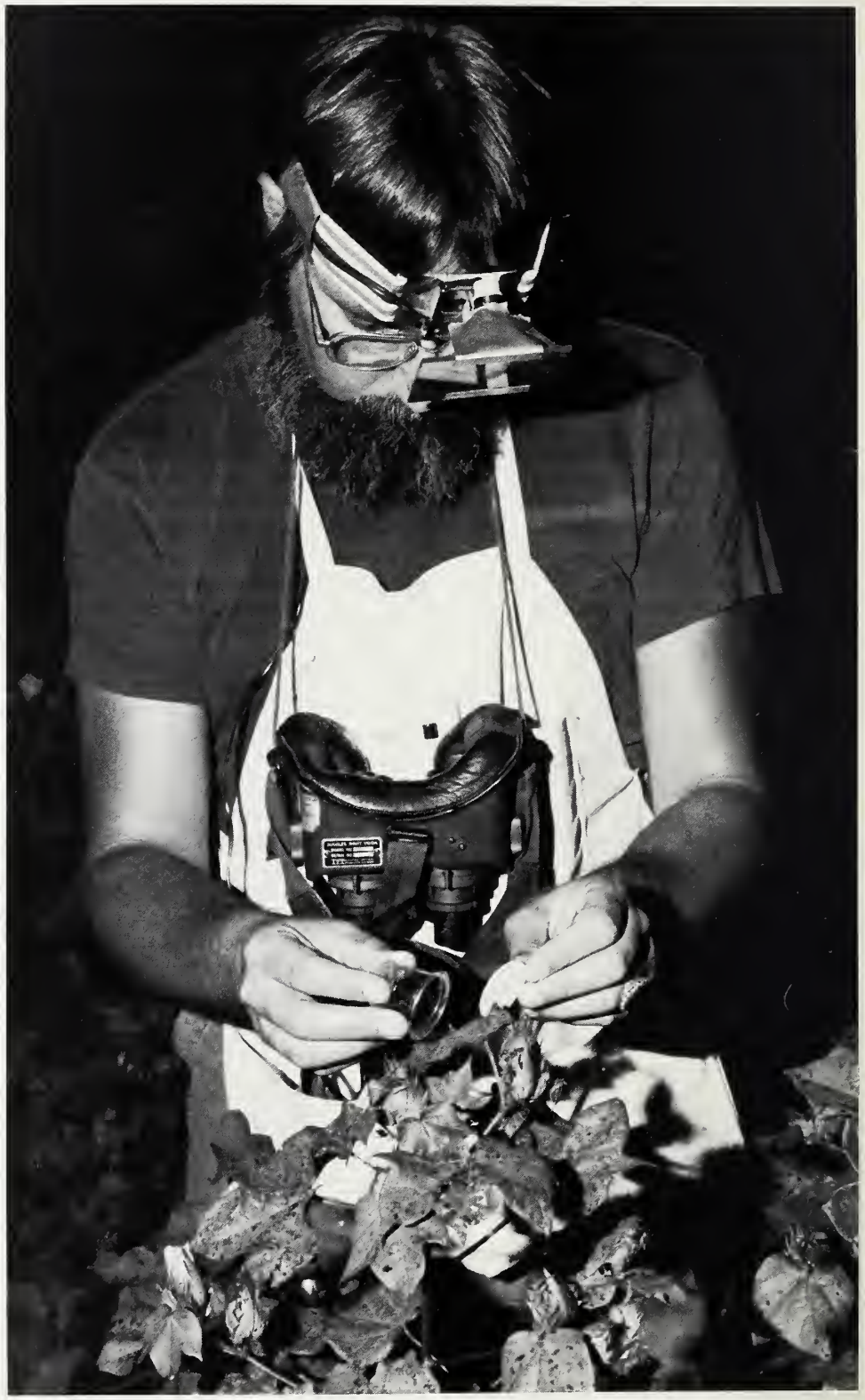
Confusing and Killing Cotton Pests

Spending sleepless nights in cotton fields watching the nocturnal routines of night-flying insects, an ARS scientist may have developed an idea that could change the direction of some insect control practices.

The idea is called an attracticide.

Entomologist and "insect behaviorist" Pete D. Lingren, Phoenix, Ariz., has been studying the habits of night-flyers with the use of headlamps, night vision goggles, TV cameras, and in some instances, radar.

Those studies have led to a fairly good understanding of the nocturnal behavior of several insect species, which has resulted in the redesign and improvement of insect traps and only recently in the development of the attracticide, which, says Lingren, "Could soon drastically change the insect control picture."



Above left: With "claspers" extended and wings fluttering, a pink bollworm adult tries to mate with an attracticide-treated cotton leaf. Within hours the bollworm will die from contact with the treated leaf. (1181X1477-8)

Above: To determine the killing power of the treated leaves, technician Terry Meyers collects pink bollworm males as they land on the leaves in confused mating attempts. The night-vision goggles around his neck are used to study nocturnal insect habits. (1181X1477-31)

In addition to the use of insecticides and other practices, cotton farmers and insect control concerns have been using synthetic sex pheromones to confuse male pink bollworms and disrupt mating. Tiny plastic tubes impregnated with pheromones have been distributed in cotton fields so that male pink bollworms are unable to locate females because their receptors are overwhelmed by the abundance of the sex attractant. The practice in some instances has reduced pink bollworm populations and is becoming a part of integrated pest management schemes.

Lingren, in observing the reactions of male pink bollworms to several slow-release pheromone formulations, including the plastic tubes, saw that in many instances the male pinkies tried to mate with leaves of treated cotton plants.

That observation led to Lingren's mixing the pheromone into a commercially available substance—called an adjuvant—and lacing the mixture with an insecticide.

The adjuvant was originally developed by coworker Randy Bell for use with microbials—microorganisms that are pathogenic to specific insects—and consists of cottonseed flour, cottonseed oil, sugar, and a surfactant. It is itself a feeding stimulant and is tasty to pink bollworm adults of both sexes. The oil helps to extend the effective life of the pheromone and the overall formulation appears to prolong the life of the insecticide.

The males, lured to the pheromone in the attracticide, come in contact with the insecticide through mating attempts, become nonfunctional in a short time, and die within a few hours. Some females are also killed because they crawl around much of the night on the upper portions of cotton plants and may ingest the insecticide when they eat the sugar in the adjuvant.

Small field-scale tests showed 90 percent kill of the males contacting the attracticide, and in one larger test the substance suppressed high-density population below economic levels for 3 weeks. Other tests using the material in traps for 9 weeks showed the attracticide to be as good as anything currently available for pink bollworm.

Many night-flying cotton pests feed, lay eggs, rest, secrete pheromones, and mate on the upper portion of cotton plants during certain periods of the night. In addition, adults of such species as the tobacco budworm emerge from pupae at night and move to upper portions of cotton plants where they are immobile and vulnerable for several hours. Their larvae also move at night from the bolls to the top of the plant where they shed and eat their exoskeletons. They stay at the top of the plant for at least an hour.

That type of behavior, observed during long hours of the night, leads Lingren to recommend concentrating the attracticide at the top of the plant in droplet sizes larger than those of most insecticide applications. It might be possible to strip-treat fields since the formulation attracts males to the source.

Lingren cites the following benefits of an attracticide technology:

- It should provide comparable or better control than current methods, with less pesticide, because it attracts and kills males in the relatively small upper portion of the plant.
- Since it is aimed primarily at adults, the attracticide should kill adults before progeny—which cause the damage—are produced.
- It should have a tendency to act like the pheromones used in confusion techniques and cause some mating disruption.
- Since females crawl around and over the tops of the plants where the attracticide is concentrated, it should result in a greater degree of female kill than most conventional insecticide applications.
- It should fit in well with integrated pest management efforts.
- It should allow several species to be attacked with several modes of control through one delivery system.

Lingren is working with ARS entomologists Dan Wolfenbarger and Jim Raulston at the Cotton Insects Research Laboratory, Brownsville, Texas, on possible uses of the attracticide on the boll weevil and other cotton insect pests. More research is still needed on insect behavior, good slow-release formulations for other species, proper selection of adulticides, and application techniques.

Concurrently, at the Phoenix laboratory, Lingren is continuing his study of insect behavior, Louis A. Bariola is seeking to improve the selection of insecticides, and Randy Bell is working on proper application procedures for the attracticide.

Tobacco budworm adults mate on an untreated cotton leaf. (1181X1475-13)

Pete D. Lingren is located at the Western Cotton Research Laboratory, 4207 E. Broadway Road, Phoenix, AZ 85040.—(By Paul Dean, Oakland, Calif.) ■

Freezing Potato Shoot Tips

Above left: In a growth chamber, Towill examines plantlets of various potato cultivars and species. The plantlets are used in studies of cryogenic (low-temperature) preservation. (0382X249-34a)

Above: Plant physiologist Leigh E. Towill exposes excised potato shoot tips to low temperatures with controlled cooling rates. Seven species have been successfully preserved. (0382X248-6a)

Strains of potatoes in extensive germplasm collections may be preserved more conveniently than they are now when techniques in cryogenics and tissue culture of plants are refined.

The basic methods were developed for cooling isolated shoot tips of a nontuber-bearing potato relative to -196° C and then thawing and observing regrowth. With this procedure ARS plant physiologist Leigh E. Towill has had good success with several potato varieties.

"Some day we hope to store shoot tips of potatoes routinely for decades, but first we need to improve our techniques to enhance survivability. Working with the nontuber-bearing plants and shoot-tip systems gave us some insights on improving cryogenics of tuber-bearing plants," Towill says.

Towill has even had success with his techniques on shoot tips from several other potato species.

By preserving shoot tips instead of potato seeds, scientists can regenerate a plant that is genetically identical to the parent plant for breeding, genetic, pathological, entomological, and physiological studies.

Also, the shoot tips are obtained from plants that are free of the viruses that decrease yields of many potato varieties. If plants that are being grown in a germplasm collection become diseased, heat treatment followed by shoot-tip propagation is a means of replacing them with disease-free stock.

Towill's successful cryogenic techniques involve carefully worked out cooling and warming rates to avoid formation of intracellular ice. To avoid further damage, he incubates the shoot tips in a medium that is 10 percent dimethylsulfoxide (DMSO) for 1 hour before cooling them.

"We don't know how cryoprotectants such as DMSO function, but they are necessary to obtain survival at very low temperatures," Towill says.

Leigh E. Towill is located at the Department of Horticulture, University of Wisconsin, Madison, WI 53706.—(By Ben Hardin, Peoria, Ill.) ■

Egusi Seed for Protein

Development of indigenous egusi seed could help correct protein deficiency in countries where protein malnutrition is a problem.

Egusi, a melon-type fruit about the size of a cantaloupe, whose bitter flavor makes it inedible, is grown only for its seed. However, despite its use as a food commodity, very little nutritional information about the seed has ever been published.

ARS scientists at the Southern Regional Research Center in New Orleans, La., are studying the nutritional and chemical properties of egusi seed and its suitability for use in foods to determine its potential as a source of vegetable protein.

John P. Cherry, research leader for the study, believes egusi seed will become important in the structure and composition of foods, in addition to its use as a source of protein.

Cherry and E.N.T. Akobundu (then a graduate student at Louisiana State University and now living in Akure, Nigeria) began their analysis of egusi seed in 1980. The seeds were obtained from a market in Umuigu Oboro, Nigeria.

"Plant proteins are desperately needed in those countries where average protein intake is less than required for good nutrition and where cultural food selection minimizes use of available protein sources," says Cherry.

He maintains that production of high-protein food from native raw materials could improve diets in countries where large portions of the population suffer protein malnutrition.

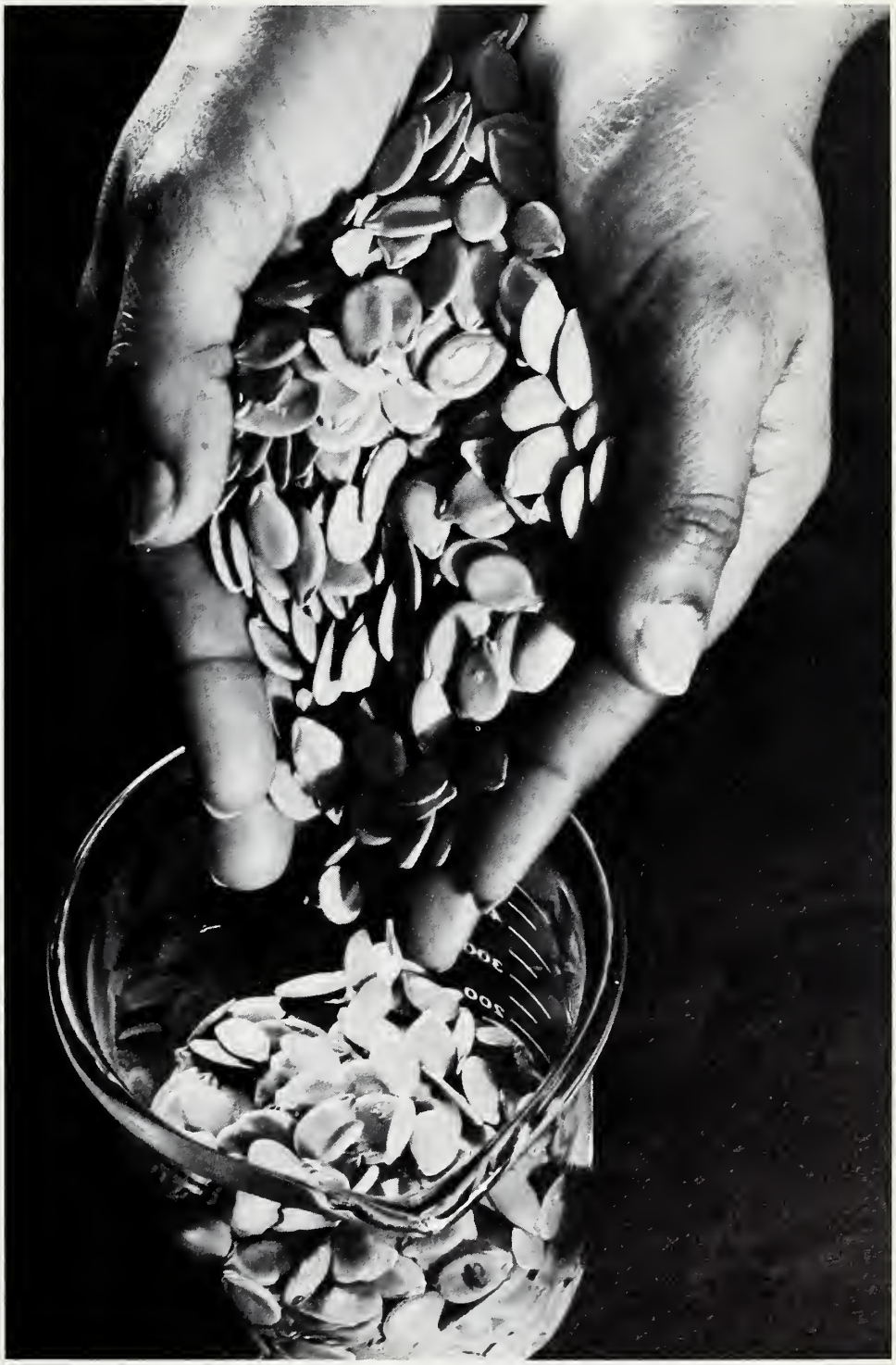
The protein content of the dehulled egusi seed was found to be better than 28 percent. The seed also contains high levels of oil (52 percent) and is low in carbohydrates (8.2 percent).

Most of the analyses were done on low-hull flour, the most likely form for commercial development.

Cherry found that defatted flours contain important amounts of various vitamins, especially thiamin and niacin, which would contribute significantly to the diet of those living in developing countries.

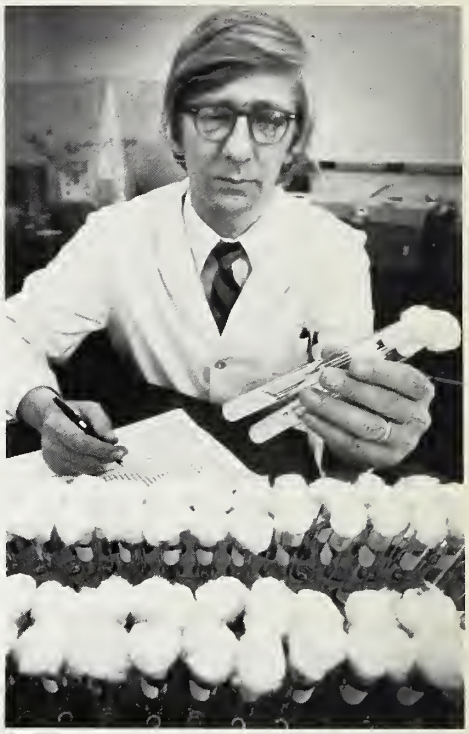
"Egusi seed flour contains excellent quantities of the major nutrients, oil, and proteins. The essential amino acids in the proteins of the flour make it a good vegetable protein," he says.

The use of egusi seed flour as a source of calcium and niacin could improve diets considerably in regions of lower Africa where little milk is consumed but egusi cultivation thrives. Test results further support the contention that components of egusi seed have good potential for use in a number of food formulations.



Seeds from the melon-type egusi fruit show potential as a protein source for developing countries. (0582W442-12a)

John P. Cherry is located at the Southern Regional Research Center, P.O. Box 19687, New Orleans, LA 70179.—(By Neal Duncan, New Orleans, La.) ■



A yeast called "odd" by French scientists who sent it to the ARS Culture Collection 25 years ago now converts xylose, or wood sugar, to ethyl alcohol in continuous process studies at the Northern Regional Research Center (NRRC), Peoria, Ill.

Rodney J. Bothast, research leader of the ethanol process studies, says xylose constitutes 10 to 25 percent of annual crop residues and processing wastes. Successful xylose fermentation could add more than 4 billion gallons to the estimated volume of fuel alcohol that could be produced every year from straw, stalks, hulls, cobs, other plant residues, and wastes from processing such as making paper.

In addition to serving as fuel, ethyl alcohol can replace petroleum products as a solvent, as an ingredient in pharmaceuticals, detergents, cosmetics, antifreeze, and in manufacturing rubber, plastics, coatings, and intermediates, which are starting materials for still other products.

Left: Cletus P. Kurtzman, research leader, holds a few of the more than 14,000 yeasts in the ARS Culture Collection. (0382X142-11)

Above: Kurtzman selects yeasts that can grow on various sugars and other organic compounds. Promising yeasts are tested further for their economic feasibility. (0382X145-8a)

Until a systematic search of the Culture Collection turned up *Pachysolen tannophilus*, no yeast was known to convert xylose directly to ethanol. The Collection holds more than 75,000 cultures of yeast, molds, bacteria, and other microorganisms important to agriculture and other industries. Maintained at the NRRC since 1940, it preserves pure, viable cultures for international research.

Pachysolen is not exactly a xylose specialist, however. Like most yeasts, it prefers glucose, says Cletus P. Kurtzman, Culture Collection research leader, but unlike them, it can ferment xylose.

Glucose, sometimes called corn sugar, is the building block of starch cellulose (see *Agricultural Research*, May 1980, p. 10). Xylose suger is found in hemicellulose, which accompanies cellulose in woody or fibrous skeletal parts of plants. By Webster's definition, xylose is "not fermentable with ordinary yeasts."

By definition of the French who found it, *Pachysolen* is no ordinary yeast. Although scientists have demonstrated that it converts xylose to ethanol, they are still trying to explain the exact chemical mechanism of the conversion.

French scientists isolated the yeast in 1957, after they found it in leather-tanning liquors that contain extract from the wood of chestnut trees. During the next 4 years, studies of the NRRC Culture Collection confirmed the characteristic that had led the French to call the organism "remarkable"—a thick-walled tube that grows from a vegetative cell. The thickened walls refract light and glow under a light microscope. "This makes *Pachysolen* identifiable even at moderate magnifications," Kurtzman says. The tip of the tube enlarges to form a thin-walled sac, or ascus, where spores form and are released.

In 1958, while studying the evolution of yeasts that produce a gum, zymologist Lynferd J. Wickerham (now retired) and Kermit A. Burton examined *Pachysolen* and suggested a symbiotic relationship between the yeast and bark beetles. As a relative of primitive yeasts that produce phosphomannan, the gelatinous gum, the *Pachysolen* cells "adhere to bark beetles as they make holes through the bark of trees, thereby carrying the yeasts to their substrate, sap."

"The symbiosis is one of long standing and good balance... In return for transportation... the yeasts may serve as food for beetles and their larvae... They form walls around the galleries, which prevent drowning of the beetles by blocking the flow of sap into these chambers."

Chemical engineer Patricia J. Slininger and chemical engineering technician Adolph A. Lagoda prepare a 20-liter fermentor for growing *Pachysolen* cells. (1281X1554-15)

Kurtzman selected *Pachysolen* and some other likely candidates in the Culture Collection when Bothast and his research team were looking for a xylose fermentor in 1979. Bothast and lab technician James E. VanCauwenberge then grew the yeast in fermentors to learn if it would produce enough alcohol to have industrial process potential. When they determined that it did, they turned it over to Patricia J. Slininger, a chemical engineer, for processing experiments.

In batch process studies, she found that *Pachysolen* grows and produces alcohol best at 32° C (84-85° F). It needs oxygen to grow but not to produce alcohol. "Alcohol production can occur in the absence of growth," she says.

She grew the yeast in acidic solutions (pH 2.5) containing minerals, nitrogen, and 50 to 250 grams of xylose per liter of solution. "Both growth and alcohol production were maximum when the initial xylose concentration did not exceed 50 grams per liter," she says. "The yeast appeared to be inhibited by ethanol concentrations above 20 grams per liter." From 100-pound batches of xylose, *Pachysolen* produces 4 to 5 gallons of ethyl alcohol.

Slininger says that *Pachysolen's* rapid production of substantial amounts of ethanol "disproves the belief that yeasts cannot convert aldopentoses (5-carbon sugars) to ethanol."

According to Bothast, the batch process is "a way to produce ethanol from xylose with the techniques and equipment commonly available in the fermentation industry." The continuous process

Microbiologist Rodney J. Bothast adds a solution of xylose to a pilot fermentor. The batch process allows ethanol to be produced with equipment that is commonly available in the industry. (0481X448-34a)

system now under study at the NRRC holds promise as a way to make alcohol from the waste stream from a papermill or by-product of other processing—"the continuous conversion of a continuously produced waste," Bothast says.

To prepare *Pachysolen* for the continuous processing studies, Slininger mixed cells of the yeast with a gel, then added this suspension by drops to a solution containing calcium chloride. This technique, studied earlier by NRRC chemist John E. McGhee, causes the gel drops, containing yeast cells, to form beads that resemble tapioca pellets.

The technique provides a dense cell population. Slininger points out that since *Pachysolen* requires oxygen for reproduction but not for fermentation, either a dense population must be provided to start the fermentation, or oxygen must be supplied so the starting cells can reproduce a dense population.

The yeast cells, packed in the gel beads, ferment xylose to alcohol in stirred flasks. "Fresh medium was continuously supplied to a well-stirred fermentor as fast as ethanol-bearing effluent was removed," Slininger says.

She found that the yeast cells retained at least half of their original productivity after the process continued 26 days. Alcohol yields from the continuous process are about the same as batch process yields.

In related experiments at NRRC, scientists are teaming *Pachysolen* with another yeast, *Saccharomyces uvarum*, to determine if glucose and xylose from plant residues can be fermented simultaneously.

Slininger loads a small continuous fermentor with tapioca-sized pellets of *Pachysolen* cells. (1281X1556-17a)

The scientists ground and cooked the straw, making pulp by two processes similar to those used in making paper. They divided the pulp into cellulosic and hemicellulosic fractions, each containing pulping by-products as well as sugars.

Saccharomyces fermented glucose obtained from the cellulose to alcohol, and *Pachysolen* converted xylose from the hemicellulose to alcohol. Research leader Robert W. Detroy says the pulping by-products prevented the yeasts from producing expected yields of alcohol, but the study demonstrates that direct, total fermentations of crop residues and processing wastes are possible

Rodney J. Bothast, Cletus P. Kurtzman, and all other scientists named in the article are located at the Northern Regional Research Center, 1815 North University, Peoria, IL 61604.—(By Dean Mayberry, Peoria, Ill.) ■

Salt Control for the Grand Valley

Efforts are underway to reduce the estimated 585,000 to 720,000 tons of salt that western Colorado's Grand Valley contributes annually to the Colorado River.

These salts, from sodium chloride (common table salt) to more complex ions, reduce water quality for downstream users and are a growing concern for irrigators and urban users in Mexico and the southwestern United States.

The Grand Valley salt load amounts to about 10 tons annually for each acre irrigated. Damages to downstream water users are currently estimated at $43 for each ton of salt.

About 90 percent of the salt added to the Colorado River as it flows through the Grand Valley is the result of irrigation in the Valley. Roughly one-third comes from each of the following: water seepage from main canals, water seepage from laterals that deliver water from canals to farms, and farm practices.

Such massive damage justifies irrigation improvements in the area. The U.S. Department of Agriculture's (USDA) Agricultural Research Service, Soil Conservation Service (SCS), and Agricultural Stabilization and Conservation Service (ASCS), and the U.S. Department of Interior's Bureau of Reclamation (USBR) are coordinating a salinity control project in the Grand Valley. The USBR proposes o line with concrete about 185 miles of existing canal, and with the assistance of USDA, to line or replace with pipe about 450 miles of open laterals.

In addition, SCS will provide technical assistance and ASCS will provide cost-share funds to line or place in pipe 655 miles of existing on-farm head ditches, improve the grade of 17,000 acres of irrigated cropland, install 55 miles of drainage conduit, and install drip or sprinkler irrigation systems on 800 acres.

One of the requirements of ASCS cost-sharing of on-farm improvements is that irrigation systems be automated or semiautomated. These systems must be designed to operate at least 24 hours, changing sets as necessary, without manual labor. Automated irrigation was pioneered by ARS agricultual engineers Howard R. Haise (retired) and E. Gordon Kruse, of Fort Collins, Colo., and Allan S. Humphreys, of Kimberly, Idaho. This work is being continued at Fort Collins by Kruse, Harold R. Duke and M.L. "Bud" Payne.

Some of the systems being used in the Grand Valley are concrete-lined ditches with timed check gates controlling water-flow through portholes; buried pipelines with automated alfalfa valves (developed by the ARS Irrigation Management Group, Fort Collins); or gated pipe with mechanical timers to control check valves (developed by Humphreys). ARS soil scientist William D. Kemper, Snake River Conservation Research Center, Kimberly, has installed a system called Cablegation in the Grand Valley (see *Agricultural Research*, June 1981, p. 7). He will continue to evaluate its effectiveness.

Furrow and corrugation irrigation systems are needed for the Grand Valley because of the generally fine-textured soils found there. Surface systems eliminate the need for sprinkler irrigation and thereby avoid its high energy requirements. Also, the small farm and field sizes are not suited to the center-pivot sprinklers so popular in other areas. These systems would also require very careful design and management to prevent excessive surface runoff. Properly designed and managed surface irrigation systems can achieve efficiencies equal to those of sprinklers on suitable fields.

Another benefit of automating surface irrigation systems will be labor requirements. The labor required to set siphon tubes in ditches is about 1 hour per acre per irrigation. Automation can reduce this to a fraction.

ARS scientists stationed at Fort Collins, Kimberly, and Phoenix, continue to seek improvements for existing systems as well as to develop new ones that will be suited to the Grand Valley.

Currently a major research effort is directed at using laser-controlled land leveling machinery to create "dead level" basins for surface irrigation. With level basin irrigation, a large stream of water enters the basin and quickly spreads out to a uniform depth for even infiltration. There is no surface runoff because all water remains contained in the basins. With large water streams and short application times, level basin irrigation is easily automated.

In other Grand Valley studies, Kruse, with ARS soil scientist Daniel F. Champion and Colorado State University agricultural engineer David A. Young, is studying the effects of high water tables on crop production and irrigation water requirements. Colorado State University and ARS scientists are cooperating on research to design and operate pipelines that will transport sediment-laden water without clogging, and on research to determine the potential for minimum tillage of row crops under surface irrigation.

E. Gordon Kruse, Harold R. Duke, and M.L. Payne are located at the Colorado State University, Agricultural Engineering Research Center, Fort Collins, CO 80523. Allan S. Humphreys and William D. Kemper are located at the ARS Snake River Conservation Research Center, Box 186, Kimberly, ID 83341.—(By Dennis Senft, Okaland, Calif.) ■

Exploding Blueberries

Using a continuous explosion-puffing machine to dehydrate blueberries, scientists have found a way to increase the shelf life and shippability of this special fruit and improve its after-storage flavor and texture.

The world's first explosion-dryer for fruit and vegetables, USDA's prototype, was designed and built several years ago by an ARS research team led by mechanical engineer Wolfgang Heiland at the Eastern Regional Research Center (ERRC), Philadelphia, Pa.

Explosion puffing is suitable for drying many other fruits and vegetables, and it is already used commercially with onions and carrots. Successful use of the process on blueberries is a recent accomplishment that may greatly expand markets for the popular fruit.

Almost all blueberries, wild or cultivated, grow on the North American continent. The berries are popular when in season, and in Europe and elsewhere people pay high prices for them. However, supplying enough blueberries to consumers here or abroad is difficult. The harvest season is only 6 weeks in spring. Extending the selling season by drying or freezing the berries has not been as effective with blueberries as it has been with other fruits.

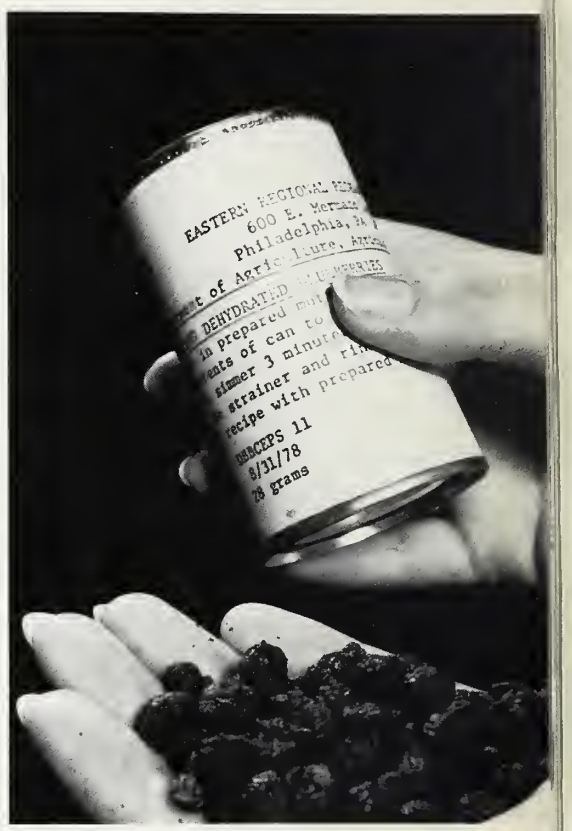
The explosion-drying process involves exerting heavy pressure on small batches of blueberries inside a heated chamber. When the pressure is suddenly released, water in the blueberries literally explodes from the fruit. Each explosion-dried batch, popping from the machine at regular beats, looks like soft, blue gravel. Explosion-dried blueberries are porous, slightly puffed, and crunchy—they're a good snack food. But adding boiling water restores the "blue gravel" to a form surprisingly close to fresh blueberries. In fact, reconstituted, explosion-dried blueberries are superior in flavor and texture not only to berries dried by other means, but also to canned and frozen berries.

ERRC chemical engineer John Sullivan says the process could solve two blueberry marketing problems. "First, growers would no longer need to sell their entire fresh blueberry crop at peak season low prices. Growers' cooperatives could explosion-dry part of their crop in order to extend sales throughout the season. Secondly, for distributors and sellers explosion-drying is a good alternative to canning or freezing."

Freezing, the most popular blueberry-preserving method, is energy intensive and expensive. In contrast, the prototype explosion-dryer saves 40 percent of the energy required by conventional food-drying systems. Also, the shelf life of frozen blueberries is only 6 months, after which they become woody or gritty, Sullivan says. Explosion-puffed berries may be rehydrated to near-fresh form even after a year or longer. They also are more durable than fresh blueberries and cost less to transport and store because they do not require refrigeration.

The Georgia Blueberry Association supplied blueberries to the ERRC for the recent explosion-drying experiments. Encouraged by the results, the Association, in cooperation with the Georgia State Coastal Plains Regional Service Commission, is building a facility that will explosion-dry blueberries for Association members.

Wolfgang Heiland and John Sullivan are located at the Eastern Regional Research Center, 600 East Mermaid Lane, Philadelphia, PA 19118.—(By Stephen Berberich, Beltsville, Md.) ■



Explosion-dried blueberries resemble blue gravel. When they are reconstituted with boiling water, their flavor is almost identical to fresh berries. (0682W551-32)

The baking characteristics of reconstituted blueberries are the same as fresh ones. (0682W552-14a)

The Beetle Who Lives with Fire Ants

What kind of creature would deliberately set up housekeeping in a fire ant's nest? A not-so-crazy beetle, known scientifically as *Myrmecaphodius excavaticollis.*

This beetle is a myrmecophile, an insect that seeks out and shares an ant's nest. To the host ants, they are definitely unwelcome guests, but the visitors have well-armored exteriors and play dead when they are attacked. The ones who survive move freely among their hosts, helping themselves to an insect smorgasbord.

Fire ants, usually the bullies of the ant world, provide a varied menu. The beetles obtain food directly from the worker ants by eating regurgitated food, and the fire ant larvae and pupae. They also feed on freshly killed or decomposed workers and raid the forager ants' pantry of collected staples—other dead insects.

In laboratory studies, the beetles were observed to mingle with their hosts without showing any signs of aggression. This could not be explained simply by the beetles' tough exterior and death-feigning behavior, and led chemist Robert K. Vander Meer and entomologist Daniel P. Wojcik to the discovery of an unusual phenomenon.

The beetles acquired their hosts' specific cuticular hydrocarbons, materials produced on the outer surface of ants. The beetles were also able to shed the hydrocarbons of one ant species, retain their own, and then acquire those of a different host species. In short, these beetles can move in on a new host colony and repeat their "freeloading" at will.

Vander Meer and Wojcik recently reported for the first time this novel insect integration method, which they call "chameleon-like chemical mimicry." How the beetles locate a potential host site and enter a colony is unknown, but the key to their acceptance may lie in the odor of the host colonies and species, composed of hydrocarbons.

Colony odor is assumed to contain innate (species specific) and acquired components. Since ants recognize each other by touching their antenna to the cuticle or outer surface of another ant, researchers believe that the ants' cuticles synthesize innate chemicals and provide a large surface for release of these chemicals.

The cuticle can also absorb the acquired components of colony odor from the surrounding environment, such as soil or plant odors in the immediate vicinity. These acquired environmental factors give each colony of the same species an odor that is distinctly its own. The innate components may add nuances to the individual colony odors.

Hydrocarbons, a class of compounds within insect cuticular components, are useful in taxonomic studies based on the chemistry of insects, and are also known to act as sex attractants and as alarm pheromones. For at least one beetle, they serve as the primary integration mechanism by producing a hydrocarbon pattern identical to its host. Hydrocarbons are also believed to play an important role in species recognition.

In laboratory studies with black imported fire ants and *M. excavaticollis,* Vander Meer and Wojcik found that the beetle acquired host-specific hydrocarbons while maintaining its own innate compounds.

"If the host hydrocarbons are acquired, then as a chameleon changes color, this multiple-host myrmecophile should be able to change its hydrocarbon pattern to match the host species," say the researchers.

The scientists tested this hypothesis by isolating beetles collected from black imported fire ant colonies for 2 weeks before introducing them into laboratory colonies of red imported fire ants. The surviving beetles (20 percent) were removed and analyzed for cuticular hydrocarbons.

The data conclusively showed that *M. excavaticollis* acquired the cuticular hydrocarbons of its new host. However, the survival rate of the beetles may have been much lower than in the field. In the laboratory colonies the beetles had no place to hide.

M. excavaticollis probably used one of its hosts as a visa to enter the United States with the accidentally imported red and black fire ants from South America as long ago as 1920, according to the scientists. The beetles have adapted very well to the southern states and have been reported in the mounds of three ant species indigenous to this country, as well as those of another imported ant.

All developmental stages of the beetles have been found within host mounds. The beetles have a continuous flight pattern with peak activity in June and July. Dissections of beetles found in light traps indicate that the females fly in all stages of ovarian development and probably breed all year long. After these dispersal flights the beetles must find a suitable host colony, which may not be the same species they left.

The research primarily adds to the basic entomologic knowledge of myrmecophile beetles and imported fire ants. Furthermore, if the beetles survived in high enough numbers to eat more fire ant larvae and pupae, they might have future bearing on fire ant control. Currently, fire ants may be controlled with toxic baits on nonagricultural or pasture land only, and only by one approved chemical compound. Several chemicals are available for mixing with water as pour-on treatments to control individual colonies. This method is not practical for large-scale imported fire ant control

Robert K. Vander Meer and Daniel P. Wojcik are located at the Insects Affecting Man and Animals Research Laboratory, 1600 S.W. 23rd Dr., P.O. Box 14565, Gainesville, FL 32604.—(By Peggy Goodin, New Orleans, La.) ■

Two Rift Valley Fever Vaccines

Scientists have completed testing two vaccines that may control the spread of Rift Valley Fever, a deadly viral disease of livestock. This disease also can infect people. Rift Valley Fever has spread from the sub-Sahara to Egypt, where thousands of people and livestock animals have contracted the disease. It is feared that the virus may spread to the Mediterranean and to other regions of the world. To date, Rift Valley Fever has not appeared in the United States.

"If by using vaccines the disease can be controlled in livestock, then the spillover into human populations can also be controlled," says Jerry S. Walker, a scientist at the ARS Plum Island Animal Disease Center, N.Y.

The vaccines were made by growing the virus in tissue cultures and then killing the virus with formalin. Although the first vaccine that the scientists tested was originally developed for use in humans, the second vaccine, which was tested in early 1980, was specifically developed for use in livestock.

The livestock vaccine was developed by the U.S. Army Medical Research Institute of Infectious Diseases in Frederick, Md., and produced under contract with the Salk Institute facility in Swiftwater, Pa.

Plum Island scientists have found that two inoculations followed by a yearly booster are necessary to protect livestock fully from Rift Valley Fever. South African and Rhodesian scientists, working independently, also found that two initial inoculations are needed to protect livestock.

Plum Island researchers exposed five unvaccinated pregnant cows to the live virus. One cow died and another aborted. The infection killed the fetuses of the other three cows. The scientists also exposed 26 vaccinated cows to the virus, 25 of which were pregnant. All of these cows lived, but one aborted its calf. Upon post mortem examination, 22 cows were found with live fetuses, and 2 with dead fetuses.

The disease is spread primarily by mosquitoes, and sheep are its principal host. When sheep become infected, almost 100 percent of pregnant ewes abort their lambs; adult sheep have death rates as high as 60 percent. In cattle the disease is not quite so deadly; about half of infected pregnant cows abort their calves, and the death toll for adult cattle is 20 percent, considerably lower than that for sheep.

"In many parts of Africa, not only is the disease devastating flocks of sheep, goats, and cattle needed for food and clothing, but the fever is also taking a heavy toll on cattle used for power and transportation in farming," says Walker.

Livestock that contract Rift Valley Fever also suffer liver failure, causing the infected animals to decompose quickly after dying. This makes the disease difficult to verify.

The virus was epidemic among the people of Egypt in 1977, resulting in some deaths. The disease recurred in 1978 and 1979. People are infected by mosquito bites, handling infected meat, or inhaling virus-contaminated air. Symptoms resemble those of the flu in more than 99 percent of the clinical cases, but the fever can cause hemorrhaging, loss of vision, hallucinations, and encephalitis, which in turn can result in neurological damage.

Jerry S. Walker is located at the Plum Island Animal Disease Center, P.O. Box 848, Greenport, Long Island, N.Y. 11944.—(By Darien Small, Beltsville, Md.) ■

Deep Freezing Hop Pollen

Liquid nitrogen storage, a technique already used in medicine and other sciences, has now been used by an ARS geneticist to preserve hop pollen for more than a year with no significant loss in fertility.

According to plant geneticist Alfred Haunold, Corvallis, Oreg., hop pollen is rarely fertile after a year of conventional refrigeration.

Working with ARS agronomist Phillip C. Stanwood, Fort Collins, Colo., Haunold compared the fertility of three batches of hop pollen stored for a year—the first batch in a household refrigerator, the second in a freezer set at -18° C (about 0° F), and the third in liquid nitrogen at -196° C (about -321° F)—to that of fresh pollen.

"Pollen stored in the freezer was surprisingly good," says Haunold, "but not as viable as fresh pollen. Pollen stored in the refrigerator was not viable at all. Pollen stored for a year in liquid nitrogen was equal in fertility to fresh pollen."

Hops are the main flavoring ingredient in beer, and the United States is the world's leading hops producer. In 1980, this country produced nearly one-third of all the hops grown in the world and exported more than half of these to about 70 countries.

The development of new hop varieties with superior agronomic qualities has been hampered by the flowering characteristics of hop plants.

"Male and female hops are separate plants with sometimes drastically different flowering dates among genotypes," explains Haunold. "A cross between an early flowering female and a late flowering male has often been difficult." Being able to keep hop pollen viable in storage would help overcome this difficulty and benefit breeding programs.

Haunold warns that precautions must be taken before storing hop pollen in liquid nitrogen. Primarily, the moisture content of the pollen must first be reduced to about 10 percent. "Otherwise," he says, "the water freezes and the pollen grains clump and become unusable."

Alfred Haunold is located in Agriculture Hall, Room 37, Oregon State University, Corvallis, OR 97331.—(By Lynn Yarris, Oakland, Calif.) ■

Fertile Soils Need Less Nitrogen

Fertile soils such as these in the Platte River Valley of Nebraska may release enough nitrogen to plants during the crop season that fertilizer applications can be reduced without loss of yield. (676X763-4a)

Recommended nitrogen fertilizer rates for fertile soils may be too high unless soil test laboratories take into account the conversion of nitrogen in soil organic matter into mineral form that occurs throughout the crop season.

Hall County, in Nebraska's Platte River Valley, is one of the areas where that conversion, called mineralization of nitrogen, is high. There, mineralization may produce about 280 pounds of nitrate-nitrogen per acre, the kind of nitrogen needed by plants. ARS soil scientist James S. Schepers, Lincoln, Neb., bases that estimate on his combination of laboratory and field studies.

Schepers says that slashing fertilizer rates in many cases could not only save farmers money but it could also reduce pollution of groundwater with nitrates.

Nitrate concentrations in the groundwater in Hall County range between 15 to 30 parts per million (ppm) in most samples. The U.S. Public Health Service has set 10 ppm as the maximum level allowable in drinking water for infants and small children.

The ARS soil scientist says leaching of excessive amounts of nitrates into the groundwater normally has been blamed on 30 years of excessive fertilization and poor irrigation management. But mineralization of nitrogen in organic matter may lead to much of the nitrate leaching.

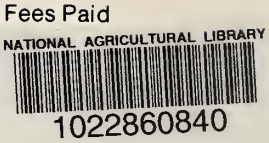
In his studies on field plots, Schepers measured organic matter content and nitrate levels throughout the soil profile, as well as temperature and moisture levels in the profile, during two crop seasons. By comparing these data with laboratory data, he found it feasible to predict nitrogen mineralization using a computer model developed by University of Nebraska agricultural engineer Darrell G. Watts.

The model simulates water percolation and nitrogen leaching. Soil testing laboratories might use some aspects of this model to estimate the amount of nitrogen that could be supplied to a crop through mineralization, Schepers suggests.

Reductions in the recommended amounts of nitrogen fertilization may occur as the decontrol of natural gas prices causes nitrogen fertilizer prices to increase. The Fertilizer Institute estimates nitrogen fertilizer prices could increase to five times their present level by 1985.

Although there are refinements to be made on the computer program, farmers in the Platte River Valley are profiting from an awareness of research that Schepers and his colleagues are conducting. Corn grown without nitrogen fertilizer for 2 years in succession on highly fertile demonstration plots yielded as much as corn grown where 200 pounds of fertilizer nitrogen per acre had been applied.

James S. Schepers is located at the University of Nebraska East Campus, Keim Hall, Rm. 113, Lincoln, NE 68583.—(By Ben Hardin, Peoria, Ill.) ■



Agrisearch Notes

Standing Straw Stubble Conserves Soil Moisture

Leaving as much straw stubble as possible upright conserves the maximum amount of soil moisture during summer fallow, when no crop is grown on fields. Standing stubble deflects winds above soil surfaces while the land is fallow and helps to trap drifting snow in winter. Erect stubble also provides shade during summer and cools the soil surface, conserving moisture, says ARS soil scientist Darryl E. Smika, Akron, Colo.

Summer fallow is used extensively on the U.S. Great Plains. The practice conserves enough soil moisture for a crop to be grown every other year.

Farmers can ensure maximum standing stubble by adjusting combine cutter bars to clip just the grain heads and by avoiding unnecessary trips across harvested fields. Weed control is also a must.

Smika says that 12-inch grain stubble could withstand 13-mile-per-hour winds without significant wind movement at the soil surface. When stubble was 24 inches, winds up to 35 mph were deflected from the soil surface, thus decreasing the potential for soil moisture loss.

Leaving soil bare during fallow, which results when farmers use extensive mechanical tilling to control weeds, was the worst way to conserve soil moisture. Only a slight breeze removed soil moisture from these lands. Flattened stubble on the soil surface was also shown to be a poor water conservation practice.

Darryl E. Smika is located at the Central Great Plains Research Station, P.O. Box K, Akron, CO 80720.—(By Dennis Senft, Oakland, Calif.) ■

Hemoparasite Lab Moved to Northwest

In a move to bring the research to the area where it is most needed, ARS has relocated its Hemoparasitic Disease Research Unit (HDRU) from Beltsville, Md., to the Pacific Northwest.

Hemoparasites are microorganisms that invade the red blood cells of animals, causing anemia, and in some cases, death. The most common and widespread of these parasites is *Anaplasma*. Another troublesome hemoparasite, *Babesia*, was believed to be eradicated but is now making a comeback.

Also called gall sickness, anaplasmosis mainly affects cattle—though sheep, deer, elk, and buffalo are also vulnerable—and is a serious problem in southern Idaho, eastern Oregon, northern Nevada, California, and parts of the Southeast. Babesiosis affects dogs, cats, goats, and sometimes even people, in addition to meat and dairy animals. However, in cattle it seems to occur only sporadically along the Texas-Mexico border.

Ticks, biting flies, and humans (through sloppiness during dehorning or injection operations) are the known vectors of hemoparasites. Only a tiny amount of infected blood is needed for transmission. Adult cattle infected with anaplasmosis may die, but infected calves usually recover and become resistant. These animals generally grow up and appear and perform as if healthy, but they remain anaplasmosis carriers: if they come into contact with a herd of nonresistant adult cattle and a vector is present, herd mortality can be 30 percent or greater.

The staff of the HDRU has been assigned to three locations. The group's research leader, hemoparasitologist Kenneth L. Kuttler, along with microbiologist Willard L. Goff and zoologist Lynn D. Winward, will be located at Pullman, Wash., doing basic immunity studies in cooperation with Washington State University. Kuttler will divide his time between Pullman and nearby Moscow, Idaho, where along with entomologist David Stiller, he will study vector transmission in cooperation with the University of Idaho there. Michael Coan, also an entomologist, will study hemoparasitic epidemiology at the University of Idaho's Caldwell campus.

"The proximity of a large anaplasmosis endemic zone in the Pacific Northwest will facilitate new epidemiologic and control studies," says Kuttler, "and working with the universities will augment studies of basic immunology, serology, and cell culture."

Kenneth Kuttler is located at the Hemoparasitic Disease Laboratory, Washington State University, Pullman, WA 99164. —(By Lynn Yarris, Oakland, Calif.) ■